From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY



**PCT**

To:

BRUESS, Steven C.
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
ETATS-UNIS D'AMERIQUE

## NOTIFICATION OF RECEIPT OF DEMAND

(PCT Rule 61.1(b), first sentence
and Administrative Instructions, Section 601)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 21. 01. 97 |
| Applicant's or agent's file reference: 600.311WOI1 | IMPORTANT NOTIFICATION |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US 96/10252 | 07/06/1996 | 07/06/1995 |

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

03/01/1997

2. This date of receipt is:

☒ the actual date of receipt of the demand.

☐ the date on which the proper corrections to the demand were timely received.

3. ☐ This date is **AFTER** the expiration of 19 months from the priority date.

**Attention:** The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices)(Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.

☐ This notification confirms the information given in person or by telephone on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

| Name and mailing address of the IPEA | Authorized officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. (+49-89) 2399-0, Tx: 523656 epmu d<br>Fax: (+49-89) 2399-4465 | John Aitken<br>Telephone No. -27 35 |

*The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:*

IPEA/ EP

# PCT

**CHAPTER II**

## DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

| Identification of IPEA | Date of receipt of DEMAND |
|---|---|
| | |

**Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION**

Applicant's or agent's file reference: 600.311WOI1

| International application No. | International filing date *(day/month/year)* | (Earliest) Priority date *(day/month/year)* |
|---|---|---|
| PCT/US96/10252 | 07 June 1996 (07.06.96) | 07 June 1995 (07.06.95) |

Title of invention

MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE

**Box No. II APPLICANT(S)**

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

REGENTS OF THE UNIVERSITY OF MINNESOTA
Morrill Hall, 100 Church Street S.E.
Minneapolis, Minnesota 55455
United States of America

Telephone No.:

Facsimile No.:

Teleprinter No.:

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| US | US |

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHLIEVERT, Patrick M.
5305 Birchcrest Drive
Edina, Minnesota 55436
United States of America

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| US | US |

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

ROGGIANI, Manuela
100 Second Street Southeast, #803
Minneapolis, Minnesota 55414
United States of America

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| IT | US |

[X] Further applicants are indicated on a continuation sheet.

International application No.
PCT/US96/10252

**Continuation of Box No. II APPLICANT(S)**

***If none of the following sub-boxes is used, this sheet is not to be included in the demand.***

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

STOEHR, Jennifer
3981 Woodridge Circle
Vadnais Heights, Minnesota 55127
United States of America

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| US | US |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

OHLENDORF, Douglas
9397 Olympia Drive
Eden Prairie, Minnesota 55347
United States of America

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| US | US |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| | |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(i.e. country)* of nationality: | State *(i.e. country)* of residence: |
|---|---|
| | |

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US96/10252

**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BRUESS, Steven C.
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
United States of America

Telephone No.: 612/336-4711

Facsimile No.: 612/336-4751

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

**Box No. IV STATEMENT CONCERNING AMENDMENTS**

The applicant wishes the International Preliminary Examining Authority*

(i) [ ] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [X] to take into account the amendments under Article 34 of

- [X] the description (amendments attached).
- [X] the claims (amendments attached).
- [ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

**Box No. V ELECTION OF STATES**

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....

*(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)*

International application No.
PCT/US96/10252

**Box No. VI CHECK LIST**

The demand is accompanied by the following documents for the purposes of international preliminary examination:

| | | | | For International Preliminary Examining Authority use only: received | not received |
|---|---|---|---|---|---|
| 1. amendments under Article 34 | | | | | |
| description | : | 2 | sheets | ☐ | ☐ |
| claims | : | 3 | sheets | ☐ | ☐ |
| drawings | : | | sheets | ☐ | ☐ |
| 2. letter accompanying amendments under Article 34 | : | 2 | sheets | ☐ | ☐ |
| 3. copy of amendments under Article 19 | : | | sheets | ☐ | ☐ |
| 4. copy of statement under Article 19 | : | | sheets | ☐ | ☐ |
| 5. other *(specify)*: renumbered abstract | : | 1 | sheets | ☐ | ☐ |

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet
5. ☒ other *(specify)*: Acknowledgement Form (1 pg.)

**Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE**

*Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).*

By: _____
Steven C. Bruess

**For International Preliminary Examining Authority use only**

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

**For International Bureau use only**

Demand received from IPEA on:

CHAPTER II

# PCT

## FEE CALCULATION SHEET

**Annex to the Demand for international preliminary examination**

| | |
|---|---|
| International application No. PCT/US96/10252 | For International Preliminary Examining Authority use only |
| Applicant's or agent's file reference 600.311WOI1 | Date stamp of the IPEA |

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

**Calculation of prescribed fees**

| | | |
|---|---|---|
| 1. Preliminary examination fee ..... | 3000 DM | P |
| 2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)* ..... | 292 DM | H |
| 3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box ..... | 3292 DM | TOTAL |

**Mode of Payment**

| | | | |
|---|---|---|---|
| [X] | authorization to charge deposit account with the IPEA (see below) | [ ] | cash |
| [ ] | cheque | [ ] | revenue stamps |
| [ ] | postal money order | [ ] | coupons |
| [ ] | bank draft | [ ] | other *(specify)*: |

**Deposit Account Authorization** *(this mode of payment may not be available at all IPEAs)*

The IPEA/ EP [X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

| 2830 0082 | 03 January 1997 | Steven C. Bruess |
|---|---|---|
| Deposit Account Number | Date *(day/month/year)* | Signature |

| | | |
|---|---|---|
| In re application of | : | REGENTS OF THE UNIVERSITY OF MINNESOTA et al. |
| Application Serial No. | : | PCT/US96/10252 |
| Filed | : | 07 June 1996 |
| Agent Ref. | : | 600.311WOI1 |
| Title | : | MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE |

**AMENDMENT UNDER ARTICLE 34 BEFORE THE PRELIMINARY EXAMINING AUTHORITY**

European Patent Office
D-80298 Munchen
GERMANY

Dear Sir:

Prior to Examination, Applicant requests the following amendments be made to the above-identified patent application.

**IN THE SPECIFICATION**

Please find enclosed specification pages 80a and 80b regarding Indications Relating to a Deposited Microorganism that are being submitted herewith under PCT Rule 13bis(a)(i) to (iii).

**IN THE CLAIMS**

Please amend the claims by substituting original claim pages 81 through 87 with new claim pages 81 through 83. (Please also find renumbered Abstract page 84.) Claims 1-39 are replaced by amended claims 1-16. Amended claims 1-16 correspond to the original claims as follows:

| New Claim | Original Claim |
|---|---|
| 1 | 1 |
| 2 | 1,5 |
| 3 | 1, 4, 5 |
| 4-9 | 4,5 |
| 10 | 2, 3, 5 |
| 11 | 6 |
| 12 | 15 |
| 13 | 19 |
| 14 | 20 |
| 15 | 22 |

| | | |
|---|---|---|
| In re application of | : | REGENTS OF THE UNIVERSITY OF MINNESOTA et al. |
| Application Serial No. | : | PCT/US96/10252 |
| Filed | : | 07 June 1996 |
| Agent Ref. | : | 600.311WOI1 |
| Title | : | MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE |

| New Claim | Original Claim |
|---|---|
| 16 | 22 |
| canceled | 7-14, 16-18, 21, 23-39 |

## REMARKS

The claimed invention includes mutants of streptococcal pyrogenic exotoxin A (SPE-A). The mutants are substantially nonlethal compared to wild type SPE-A. Mutants can have amino acid substitutions in several secondary structure elements of SPE-A. In particular, mutants can have one to six amino acid substitutions compared with wild-type protein and at least one of the substituted amino acids is asparagine-20, lysine-157, cysteine-98 or cysteine-87.

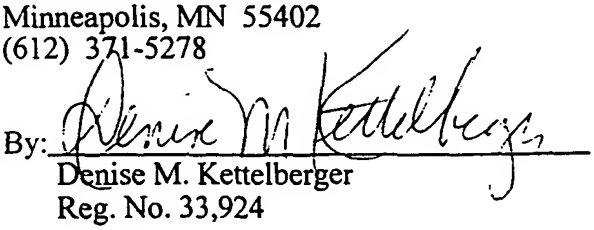
The Hartwig et al. reference cited in the International Search Report discloses 9 mutants of SPE-A, each with a single amino acid substitution. Hartwig et al. does not disclose or suggest substitution for amino acids in the claimed secondary structural elements or residues. Furthermore, Hartwig neither discloses nor suggests mutants of SPE-A with more than one amino acid substitution. Therefore, Hartwig et al. neither teach nor suggest the claimed invention.

When the Examiner takes the present application up for examination, consideration of these amendments is respectfully requested, and issuance of a favorable written opinion is earnestly solicited.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402
(612) 371-5278

Dated: 03 January 1997

By: 
Denise M. Kettelberger
Reg. No. 33,924

reference number

# INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 35, line s 8-11

**B. IDENTIFICATION OF DEPOSIT** Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, MD 20852
United States of America

| Date of deposit | Accession Number |
|---|---|
| (01.06.95) 01 June 1995 | 69830 |

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

| For receiving Office use only | For International Bureau use only |
|---|---|
| [ ] This sheet was received with the international application | [ ] This sheet was received by the International Bureau on: |
| Authorized officer | Authorized officer |

Form PCT/RO/134 (July 1992)

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description
on page 44 , lines 9-10

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, MD 20852
United States of America

| Date of deposit | Accession Number |
|---|---|
| (01.06.95) 01 June 1995 | 69831 |

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

| For receiving Office use only | For International Bureau use only |
|---|---|
| ☐ This sheet was received with the international application | ☐ This sheet was received by the International Bureau on: |
| Authorized officer | Authorized officer |

Form PCT/RO/134 (July 1992)

**WHAT IS CLAIMED IS:**

1. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least one amino acid change and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

2. A mutant SPE-A toxin according to claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

3. A mutant SPE-A toxin according to claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

4. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

5. The mutant SPE-A toxin of claim 4, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

6. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

7. The mutant SPE-A toxin of claim 6, wherein the substitution is asparagine-20 to aspartic acid.

8. The mutant SPE-A toxin of claim 6, further comprising substitution of cysteine-98, or lysine-157.

9. The mutant SPE-A toxin of claim 8, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

10. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

11. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

12. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

13. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

14. A stably transformed host cell comprising a DNA sequence according to claim 13.

15. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 11 to an animal.

16. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 11 to an animal.

**ABSTRACT**

This invention is directed to mutant SPE-A toxins or fragments thereof, vaccine and pharmaceutical compositions, and methods of using the vaccine and pharmaceutical compositions. The preferred SPE-A toxin has at least one amino acid change and is substantially non-lethal compared with the wild type SPE-A toxin. The mutant SPE-A toxins can form vaccine compositions useful to protect animals against the biological activities of wild type SPE-A toxin.

# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 600.311WO11

**Box No. I TITLE OF INVENTION**

MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE

**Box No. II APPLICANT**

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

REGENTS OF THE UNIVERSITY OF MINNESOTA
Morrill Hall, 100 Church Street, Southeast
Minneapolis, Minnesota 55455
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

**Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHLIEVERT, Patrick M.
5305 Birchcrest Drive
Edina, Minnesota 55436
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

**Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BRUESS, Steven C.
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
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United States of America

Telephone No. 612/336-4711

Facsimile No. 612/336-4751

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

**Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS**

*If none of the following sub-boxes is used, this sheet is not to be included in the request.*

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ROGGIANI, Manuela
100 Second Street Southeast, #803
Minneapolis, Minnesota 55414
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: IT

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [XX] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

STOEHR, Jennifer
3981 Woodridge Circle
Vadnais Heights, Minnesota 55127
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [XX] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

OHLENDORF, Douglas
9397 Olympia Drive
Eden Prairie, Minnesota 55347
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

**Box No.V DESIGNATION OF STATES**

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

**Regional Patent**

- [X] AP **ARIPO Patent:** KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AZ Azerbaijan, BY Belarus, KZ Kazakstan, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT, AM Armenia, KG Kyrgyzstan, MD Republic of Moldova
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT, FI Finland
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

*National Patent (if other kind of protection or treatment desired, specify on dotted line):*

- [X] AL Albania
- [X] AM Armenia
- [X] AT Austria
- [X] AU Australia
- [X] AZ Azerbaijan
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [X] BY Belarus
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- [X] HU Hungary
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- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [X] KZ Kazakstan
- [X] LK Sri Lanka
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- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova
- [X] MG Madagascar
- [X] MK The former Yugoslav Republic of Macedonia
- [X] MN Mongolia
- [X] MW Malawi
- [X] MX Mexico
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Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

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In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

**Supplemental Box** *If the Supplemental Box is not used, this sheet need not be included in the request.*

*Use this box in the following cases:*

| | |
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| *1. If, in any of the Boxes, the space is insufficient to furnish all the information:* | *in such case, write "Continuation of Box No. ... " [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;* |
| *in particular:* | |
| *(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:* | *in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;* |
| *(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:* | *in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;* |
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| *(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:* | *in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;* |
| *(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":* | *in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;* |
| *(vi) if there are more than three earlier applications whose priority is claimed:* | *in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.* |
| *2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:* | *in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.* |

CONTINUATION OF BOX V:

US which is a Continuation-In-Part of 08/480,261 filed 07 June 1995.

**Box No. VI PRIORITY CLAIM** Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

| Country *(in which, or for which, the application was filed)* | Filing Date *(day/month/year)* | Application No. | Office of filing *(only for regional or international application)* |
|---|---|---|---|
| item (1) US | 07 June 1995 (07.06.95) | 08/480,261 | |
| item (2) | | | |
| item (3) | | | |

*Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):*

[X] The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : (1)

**Box No. VII INTERNATIONAL SEARCHING AUTHORITY**

**Choice of International Searching Authority (ISA)** *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA / EP

**Earlier search** *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

**Box No. VIII CHECK LIST**

This international application contains the following number of sheets:

| | | | |
|---|---|---|---|
| 1. request | : | 5 | sheets |
| 2. description | : | 80 | sheets |
| 3. claims | : | 7 | sheets |
| 4. abstract | : | 1 | sheets |
| 5. drawings | : | 9 | sheets |
| Total | : | 102 | sheets |

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. [X] copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s)*:
5. [X] fee calculation sheet
6. [X] separate indications concerning deposited microorganisms
   X Comm. re. Sequence Listing
7. [X] nucleotide and/or amino acid sequence listing (diskette)
8. [X] other *(specify)*: PCT Transmittal
   Gen.Transmittal(in dupl.)
   X Return Postcard
   Check in amount $5352.00

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

**Box No. IX SIGNATURE OF APPLICANT OR AGENT**

*Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).*

BY: Steven C. Bruess

Steven C. Bruess

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| 1. Date of actual receipt of the purported international application: | 2. Drawings: ☐ received: ☐ not received: |
| 3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: | |
| 4. Date of timely receipt of the required corrections under PCT Article 11(2): | |
| 5. International Searching Authority specified by the applicant: ISA / | 6. ☐ Transmittal of search copy delayed until search fee is paid |

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S/N Unassigned (Based on PCT/US96/10252) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | |
|---|---|---|---|
| Applicant: | Patrick M. SCHLIEVERT, et al. | Examiner: | Unassigned |
| Serial No.: | Unassigned | Group Art Unit: | Unassigned |
| (Based on PCT/US96/10252) | | | |
| Filed: | Concurrently herewith | Docket No.: | 600.311USWO |
| (International Filing Date of 07 June 1996) | | | |

Title: MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE

CERTIFICATE UNDER 37 CFR 1.10
'Express Mail' mailing label number: EM045420175US
Date of Deposit: December 5, 1997
I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By: _____
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## PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Dear Sir:

## IN THE ABSTRACT

Please insert the attached Abstract page (page 85) into the application as the last page thereof.

## IN THE SPECIFICATION

Page 1, after the title, please insert the following paragraph:

--This application is a Continuation-In-Part of application Serial No. 08/480,261, filed June 7, 1995, now abandoned.--

Please amend the specification to include specification pages 80a and 80b filed under Article 34 with the filing of the Demand for Examination on January 3, 1997. A copy of pages 80a and 80b are enclosed.

Please also amend the specification to replace originally filed page 3 with specification pages 3 and 3a (copies enclosed) filed in Response to the Written Opinion on

August 1, 1997, and included with the Notification of Transmittal of International Preliminary Examination Report mailed September 19, 1997.

## IN THE CLAIMS

Please replace the claims with the claims (amended claims 1-23) filed in Response to the Written Opinion on August 1, 1997 (copies enclosed), and included with the Notification of Transmittal of International Preliminary Examination Report mailed September 19, 1997.

## REMARKS

A new Abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

A courtesy copy of the Notification of Transmittal of International Preliminary Examination Report is also enclosed.

The claims have been amended to bring the application into conformance with the PCT Chapter II application as amended after publication. It should be noted that the filing fees provided in connection with the application filed herewith are based on the entry of this Preliminary Amendment.

Respectfully submitted,

PATRICK M. SCHLIEVERT et al.,

By their attorneys,
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.
3100 Norwest Center
Minneapolis, Minnesota 55402
(612) 332-5300

Date December 5, 1997

By _____
Steven C. Bruess
Reg. No. 34,130

staphylococcal toxic shock syndrome toxin 1, staphylococcal enteroxtoxins A, B, Cn, D, E. G and H, and non-group A streptococcal pyrogenic exotoxins. These toxins have similar biochemical properties, biological activities and various degrees of sequence similarity.

The most severe manifestations of STSS are hypotension and shock, that lead to death. It is generally believed that leakage of fluid from the intravascular to the interstitial space is the final cause of hypotension, supported by the observation that fluid replacement therapy is successful in preventing shock in the rabbit model of STSS described above. It has been hypothesized that SPE-A may act in several ways on the host to induce this pathology. Certain single amino acid substitutions in central regions of the SPE-A molecule have been shown to affect the mitogenic activity of and binding to a HLA class II molecules by SPE-A (Hartwig et al. International Immunology 5:5, 869-875 (1993)).

SPE-A has been shown to block liver clearance of endotoxin of endogenous flora's origin, by comprising the activity of liver Kuppfer cells. This appears to cause a significant increase in circulating endotoxin, that through binding to lipopolysaccharide binding protein (LBP) and CD14 signaling leads to macrophage activation with subsequent release of TNF-α and other cytokines. Support for the role of endotoxin in the disease is given by the finding that the lethal effects of SPE-A can be at least partially neutralized by the

administration to animals of polymyxin B or by the use of pathogen free rabbits.

Another modality of induction of shock could be the direct activity of the toxin on capillary endothelial cells. This hypothesis stems from the finding that the staphylococcal pyrogenic toxin TSST-1 binds directly to the human umbilical cord vein cells and is cytotoxic to isolated porcine aortic endothelial cells.

| Applicant's or agent's file reference number | 600.311WOI1 | International application No. | PCT/US96/10252 |
|---|---|---|---|

# INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 35, line s 8-11

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, MD 20852
United States of America

| Date of deposit | Accession Number |
|---|---|
| (01.06.95) 01 June 1995 | 69830 |

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

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(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 44, lines 9-10

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

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Address of depositary institution *(including postal code and country)*

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**WHAT IS CLAIMED IS:**

1. A mutant SPE-A toxin or fragment thereof, the mutant SPE-A toxin comprising one to six amino acid substitutions and being substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin;

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

2. The mutant SPE-A toxin of claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

3. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

4. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

6. The mutant SPE-A toxin of claim 5, wherein the substitution is asparagine-20 to aspartic acid.

7. The mutant SPE-A toxin of claim 5, further comprising substitution of cysteine-98, or lysine-157.

8. The mutant SPE-A toxin of claim 7, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

9. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

10. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

11. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

12. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

13. A stably transformed host cell comprising a DNA sequence according to claim 12.

14. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 10 to an animal.

15. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 10 to an animal.

16. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least two amino acid changes and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

17. The mutant SPE-A toxin of claim 16, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains

mitogenicity comparable to that of the wild type SPE-A toxin.

18. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 16.

19. A pharmaceutical composition comprising: a mutant SPE-A according to claim 16 in admixture with a physiologically acceptable carrier.

20. A DNA sequence encoding a mutant SPE-A toxin according to claim 16.

21. A stably transformed host cell comprising a DNA sequence according to claim 29.

22. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 18 to an animal.

23. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 18 to an animal.

**ABSTRACT**

This invention is directed to mutant SPE-A toxins or fragments thereof, vaccine and pharmaceutical compositions, and methods of using the vaccine and pharmaceutical compositions. The preferred SPE-A toxin has at least one amino acid change and is substantially non-lethal compared with the wild type SPE-A toxin. The mutant SPE-A toxins can form vaccine compositions useful to protect animals against the biological activities of wild type SPE-A toxin.